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Characterisation of *S. aureus*/MRSA CC1153 and review of mobile genetic elements carrying the fusidic acid resistance gene *fusC*

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While many data on molecular epidemiology of MRSA are available for North America, Western Europe and Australia, much less is known on the distribution of MRSA clones elsewhere. Here, we describe a poorly known lineage from the Middle East, CC1153, to which several strains from humans and livestock belong. Isolates were characterised using DNA microarrays and one isolate from the United Arab Emirates was sequenced using Nanopore technology. CC1153 carries *agr* II and capsule type 5 genes. Enterotoxin genes are rarely present, but PVL is common. Associated *spa* types include t504, t903 and t13507. PVL-positive CC1153-MSSA were found in Egyptian cattle suffering from mastitis. It was also identified among humans with skin and soft tissue infections in Saudi Arabia, France and Germany. CC1153-MRSA were mainly observed in Arabian Gulf countries. Some isolates presented with a previously unknown *SCCmec*/*SCCfus* chimeric element in which a *mec* B complex was found together with the fusidic acid resistance gene *fusC* and accompanying genes including *ccrA*/*B*-1 recombinase genes. Other isolates carried *SCCmec* V elements that usually also included *fusC*. Distribution and emergence of CC1153-MRSA show the necessity of molecular characterization of MRSA that are resistant to fusidic acid. These strains pose a public health threat as they combine resistance to beta-lactams used in hospitals as well as to fusidic acid used in the community. Because of the high prevalence of *fusC*-positive MRSA in the Middle East, sequences and descriptions of *SCC* elements harbouring *fusC* and/or *mecA* are reviewed. When comparing *fusC* and its surrounding regions from the CC1153 strain to available published sequences, it became obvious that there are four *fusC* alleles and five distinct types of *fusC* gene complexes reminiscent to the *mec* complexes in *SCCmec* elements. Likewise, they are associated with different sets of *ccrA*/*B* recombinase genes and additional payload that might include entire *mec* complexes or *SCCmec* elements.

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Staphylococcus aureus (*S. aureus*) is a common coloniser, or pathogen, among humans as well as among wild and domestic animals. It can cause a broad variety of infections that include not only superficial skin and soft tissue infections (SSTI) but also life-threatening conditions such as sepsis, infective endocarditis and pneumonia. While beta-lactams are crucial for treatment, resistant strains, so-called methicillin-resistant *Staphylococcus aureus* (MRSA), were first reported nearly 60 years ago¹. Beta-lactam resistance in MRSA is caused by alternative penicillin-binding proteins encoded by different *mec* genes/alleles, out of which *mecA* is the most common and widespread one^{2,3}. The *mecA* gene is located on large and complex genetic elements, known as SCCmec (“staphylococcal cassette chromosome” or “staphylococcal chromosomal cassette” harbouring *mecA*) in which it is linked to *ccr* recombinase genes and, variably, to additional genes encoding antimicrobial or heavy metal resistance^{4–11}. Originally, MRSA was restricted to healthcare settings, but from the mid-1990s on, infections with community-acquired MRSA (CA-MRSA) were observed. Many, but not all, CA-MRSA strains carry emerging SCCmec types IV or V, as well as Panton-Valentine leukocidin (PVL; encoded by *lukS/F-PV* genes). This is a cytotoxic, pore forming toxin localized on prophages. It is associated with recurrent, chronic and/or severe SSTI as well as with rapidly progressing necrotising pneumonia. The emergence and spread of PVL-positive CA-MRSA has extensively been studied in the United States and Australia, where they are common, as well as in Western Europe, where they pose a comparatively minor problem. Less data is available for other parts of the world, but during recent years it became obvious that PVL-positive CA-MRSA are an important public health issue in Mediterranean countries, the greater Middle East, Pakistan and India. The Arabian Gulf countries are of special interest because they are a major destination for migrants, expatriate workers, tourists and pilgrims from all over the world. This might result in importation, exchange and exportation of MRSA strains epidemic to other regions of the world. Indeed, in these countries, a high degree of diversity of MRSA strains has been observed with several strains being linked to other parts of the world^{12–15}.

Since PVL is associated with clinically relevant skin conditions, topical treatments are frequently used. One option is fusidic acid, a steroid antibiotic known since the 1960s. Unfortunately, an excessive consumption of fusidic acid might quickly lead to an emergence of resistance, as it is well documented from New Zealand^{16,17}. Fusidic acid resistance is common in Middle Eastern/Arabian Gulf states, usually being due to plasmid-borne *fusB/farI*^{13,18–24} or SCC-associated *fusC*^{12,25–32}.

Here, we describe a poorly known *S. aureus* lineage from the Middle East, CC1153, to which several distinct strains from humans and from livestock belong. Most isolates identified are PVL-positive, and many are MRSA that additionally harbour *fusC*. One CC1153 strain harboured a previously undescribed SCCmec/*fus* composite element. This observation prompted Nanopore sequencing and subsequent analysis of its genome. SCCmec/*fus* are reviewed and five distinct gene clusters associated with *fusC* are defined.

Results

Description of the clonal complex. CC1153 included sequence types (ST) 1153 (1–13–1–1–124–5–3) and ST2482 (1–141–1–1–124–8–3). Possible RIDOM *spa* types are t504 (26–17–20–17–12), t903 (26–22–19–17–17–20–17–12) and t13507 (26–22–19–17–17–20–17). Isolates belonged to *agr* group II and capsule type 5. The *sasG* gene was present but *cna* and the enterotoxin homologue ORF CM14 are uniformly absent. CC1153 isolates did not harbour *egc* locus and leukocidin genes *lukD/E* were variably present.

When analysing 154 core genomic markers (Supplement 3), CC1153 appeared to be most related to CC6, CC7 and CC1290. When comparing these 154 markers, all together consisting of 124,248 nucleotides, to CC6 strain PFESA1528, (GenBank FKTB), CC7 strain TCH959 (GenBank AASB) and CC1290 strain 015H (GenBank FMMV), differences of respectively, 0.38%, 0.37% and 0.38% were noted. As comparison, for CC1 (MW2, BA000033), CC5 (N315, BA000018), CC8 (TCH1516, CP000730) and *S. argenteus* CC1850 (MSHR1132, FR821777) differences were 0.45%, 0.51%, 0.40%, and 9.18%, respectively. However, in comparison to CC6, CC7 and CC1290, different *agr* groups (*agr* II in CC1153, *agr* group I in the others) and capsule types (*cap* 5 in CC1153, *cap* 8 in the others) as well as presence of *cna* in CC6 and absence of *sasG* from CC7 were noted. As these divergent loci are localised at distant positions across the genome, an emergence of CC1153 from these lineages by a single replacement of a fragment of chromosomal DNA appears to be unlikely.

CC1153-MSSA strains. Twenty-three isolates of CC1153-MSSA were characterised (see Supplemental File 1). They originated from France (11/23), Egypt (6/23), Saudi Arabia (5/23) and Germany (1/23). The Egyptian isolates originated from cattle with subclinical mastitis, all other isolates were of human origin. CC1153-MSSA isolates, including those from cattle, are usually PVL-positive with *lukF/S-PV* being detected in 21 out of 23 isolates. Some isolates, mainly Saudi Arabian (*n* = 4) and French (*n* = 3) ones, harboured enterotoxin genes *sek* and *seq*. The staphylokinase gene *sak* and *scn* (staphylococcal complement inhibitor) were always present while only one isolate was positive for *chp* (chemotaxis-inhibiting protein). All isolates carried the penicillinase operon (*blaZ/blaI/blaR*) while other resistance genes were only sporadically found (*erm*(C), *msr*(A), *mph*(C), *aadD* and *fusB/farI*; each once in 23 isolates).

CC1153-MRSA strains. Twenty-six isolates of CC1153-MRSA were identified and characterised (see Supplemental File 1).

Nine isolates were assigned to different variants of SCCmec V or VT elements. Five of these isolates came from Kuwait, one from Riyadh, Saudi Arabia, and two from the UAE (one each from Dubai and Umm-al-Quwain) and one from an Egyptian child living in Germany. All isolates, except the oldest one (isolated in 2009³³) also carried the *fusC* gene. In two isolates, the SCCmec/*fusC* composite element was further characterised using a second microarray⁹ assigning them to SCCmec V + *fusC* (rather than to SCCmec VT + *fusC*). One isolate (from Kuwait) yielded the same pattern (with signals for *mecA*, *ugpQ*, *fusC*, *mvaS*-SCC, Q4LAG7, *ccrAA*, *ccrC*, SCCterm3, SCCterm10) as observed

in a possibly livestock–(i.e., camel–) associated CC15 strain from Saudi Arabia^{28,34}. The other one (from Germany/Egypt) yielded signals for *mecA*, *ugpQ*, *fusC*, *mvaS*-SCC, Q4LAG7, *ccrAA*, *ccrC* and SCCterm11 possibly indicating a difference affecting the SCC*mec/orfX* junction site and/or another SCC*mec/fus* subtype. Seven of these isolates harboured PVL genes. Enterotoxin genes and *chp* were not identified, but *sak* and *scn* were always present. Eight out of the nine isolates harboured *fusC*. All were positive for *blaZ/blaI/blaR*. The gentamicin resistance gene *aacA-aphD* was found in eight isolates, the tetracycline resistance marker *tet(K)* in four isolates.

Seventeen CC1153-MRSA isolates belonged to a strain which, to the best of our knowledge, carried an unknown SCC*mec/fusC* chimeric element. Three of these isolates were investigated with the second array yielding signals with *mecA*, *ugpQ*, Delta *mecR1*, *fusC*, Q4LAG7 (MSSA476), *mvaS*-SCC, *ccrA-1*, *ccrB-1* and *dcs*. This prompted genome sequencing of one isolate, henceforth designated M58 (see below). Isolates with the new chimeric element originated from Kuwait (n = 14), UAE (n = 2) and France (n = 1). All but one were positive for *lukF/S*-PV genes. Genes *sak* and *scn* were always present while enterotoxin genes were not detectable. Sixteen isolates of this strain harboured *blaZ/blaI/blaR*, and *erm(C)* was found once.

Description of the SCC*mec* element in M58. One of the seventeen isolates with an apparent unknown SCC*mec/fusC* chimeric element was subjected to genome sequencing (Nanopore) to characterise this element (see GenBank CP065857.1). Its gene content of the SCC*mec/fusC* element is summarised in Table 1, and Fig. 1 provides a graphical overview as well as a comparison to other, previously published, reference sequences.

In short, the element comprised a *mec* complex B, *ccrA/B-1* recombinase genes and *fusC*, while *tirS* (that commonly accompanies *fusC*³⁵) was absent. The gene *pls*-SCC, which normally is part of SCC*mec* I, was also absent. This constellation raises the question whether the element was derived from a SCC*mec* I element truncated by an insertion of *fusC*, or if it was a *mec* complex B element from a SCC*mec* I or IV element supplemented by *fusC* and accompanying *ccrA/B-1* recombinase genes.

The actual *mecA* allele was identical to one which is widespread in SCC*mec* IV strains including, for instance, MW2² but differing from the one in COL. The *mec* complex B was followed by some genes encoding “putative proteins” and by *ccrB-1* and *ccrA-1* recombinase genes, as it was also the case in SCC*mec* I. A closer inspection of the sequence of the genes encoding “putative proteins” and of *ccrB-1* revealed differences compared to the corresponding sequences in SCC*mec* I. The *ccrB-1* allele from SCC*mec* I in COL differed by 7.2% of its nucleotides while *ccrA-1* was too conserved to allow a meaningful analysis.

This prompted a search for possible donors of recombinase and *fusC*-associated genes. While *fusC* itself was identical to MSSA Sanger 476, GenBank BX571857.1, the surrounding region was different in both gene content (most notably, in absence of *tirS*) as well as in gene sequences (8.4% difference in *ccrB-1*). Most closely related sequences of *fusC*-associated genes were identified in the *S. aureus* CC5 strain 06BA18369, GenBank ARXY, and in the *Staphylococcus hominis* subsp. *hominis* strain NTUH-3390, GenBank KY643657.1. These strains carry Q8CU82, *tarF*-SCC, A9UFT0, Q9KX75, Q7A207, Q7A206, *ccrB-1*, *ccrA-1*, *cch-1*, DUF1413, Q83ZD5, helicase M06, Q6GD51, D3QFP0-scc, D3JCW9, *fusC*, tnpIS150, tnp_A8YYY6, Q4LAG7-SCC*fus* and *yobV* (for explanations and GenBank entries of the genes discussed, see Table 1 and Supplemental File 4). However, Q8CU82, *tarF*-SCC-1 and A9UFT0 were absent in M58. The gene encoding the putative protein Q9KX75 in M58 was virtually identical to the ones in COL and MW2 but differed from the one in 06BA18369 and NTUH-3390, GenBank KY643657.1. From Q9KX75 on downstream, however, 06BA18369 and NTUH-3390 sequences were virtually identical to the ones in M58.

The PVL prophage in M58. The sequence of the PVL prophage in the genome of M58 (CP065857.1) was identical to the one in the *S. aureus* CC1153 strain 3688STDY6124889, GenBank FQHT01000001.1. It was also identical to the PVL prophage in USA300-TCH1516, CP000730.1.

M58 was shown by a lateral flow assay to secrete detectable amounts of PVL. This was also the case for two other CC1153-MRSA-PseudoSCC*mec* [class B + *fus* + *ccrAB1*] isolates as well as for three of the CC1153-MSSA isolates.

Discussion

CC1153 and the SCC*mec/fusC* element in isolate M58. We describe a clonal complex of *S. aureus* that we identified in several Middle Eastern countries. A couple of publicly available genomes, deposited in GenBank (GenBank FQHT01000000) and/or the Short Read Archive (SAMEA2661948, SAMEA2661956, SAMEA2662240, SAMEA2662319, SAMEA2710354, SAMEA2710468, SAMEA3214613, SAMEA3448866, SAMEA3448996, SAMEA4547522, SAMN03289718) belong to it, but to the best of our knowledge, this clonal complex has not yet been reviewed. Three of these sequences originated from Thailand (GenBank FQHT01000000.1 as well as BioSamples SAMEA3448866 and SAMEA3448996), and one from the United Kingdom (SAMN03289718) while for the others, no locations were reported. An additional observation of CC1153 isolates originated from Myanmar³⁶. Our isolates were collected in the greater Middle East (Egypt and Arabian Gulf countries) and Western Europe although at least one of the European cases had connections to Egypt. There are no data confirming or explaining a discontinuous distribution in the Middle East and in South-East Asia. However, the presence of millions of expatriate South-East Asians in the Gulf countries could easily explain a transmission of a *S. aureus* lineage into either direction.

An interesting observation is the presence of CC1153 in Egyptian bovines³⁷. The detection of PVL (rather than of *lukM/lukF*-P83) and of haemolysin-beta-converting prophages in these isolates indicates a human provenance of these isolates so that the cattle probably served as sentinels for an unrecognised epidemiological situation among humans in the Nile Delta.

The majority of CC1153, including MRSA and MSSA, is PVL-positive harbouring the same prophage (in M58 and FQHT01000001.1) as other pandemic strains such as USA300.

| Gene ID | Explanation | Position in SCC (nt) | Position in genome (nt) | Length (nt) | Direction | Sequence identical to |
|----------------------|--|----------------------|-------------------------|-------------|------------------|---|
| <i>orfX</i> | (23S rRNA methyltransferase) | | 33,698–34,177 | 480 | Forward | |
| sRNA6 | (Antisense RNA associated with <i>orfX</i>) | | 33,878–34,161 | 284 | | |
| DR_SCC | Direct repeat of SCC | 1–19 | 34,159–34,177 | 19 | | |
| <i>dcs-L1</i> | Downstream constant segment, locus 1 | 20–301 | 34,178–34,459 | 282 | | MW2, BA000033 [34169:34450] |
| Q9XB68- <i>dcs</i> | Putative protein | 302–1596 | 34,460–35,754 | 1295 | Forward | Strain 21172, AFEF01000013 [388744:390039] |
| Q7A213 | Putative protein | 2011–2250 | 36,169–36,408 | 240 | Forward | COL, CP000046 [36082:36321] and MW2, BA000033 [36161:36400] |
| IR_IS431 | Inverted repeat of IS431 | 2223–2238 | 36,381–36,396 | 16 | | COL, CP000046 [36294:36309] and MW2, BA000033 [36373:36388] |
| tnpIS431 | Transposase for IS431 | 2282–2956 | 36,440–37,114 | 675 | Reverse (trunc.) | COL, CP000046 [36353:37027] |
| Teg143 | Trans-encoded RNA associated with tnpIS431 | 2987–3020 | 37,145–37,178 | 34 | | COL, CP000046 [37058:37091] and MW2, BA000033 [37137:37170] |
| IR_IS431 | Inverted repeat of IS431 | 2997–3012 | 37,155–37,170 | 16 | | COL, CP000046 [37058:37091] and MW2, BA000033 [37137:37170] |
| <i>mvaS</i> -SCC | Truncated 3-hydroxy-3-methylglutaryl CoA synthase | 3029–3381 | 37,187–37,539 | 353 | Forward | COL, CP000046 [37100:37452] and MW2, BA000033 [37179:37531] |
| Q5HJW6 | Putative protein | 3479–3709 | 37,637–37,867 | 231 | Forward | COL, CP000046 [37550:37780] and MW2, BA000033 [37629:37859] |
| <i>dru</i> | SCC direct repeat units | 3619–4056 | 37,777–38,214 | 438 | | COL, CP000046 [37690:38127] |
| <i>ugpQ</i> | Glycerophosphoryl diester phosphodiesterase | 4258–5001 | 38,416–39,159 | 744 | Forward | COL, CP000046 [38329:39072] and MW2, BA000033 [38288:39031] |
| <i>ydeM</i> | Putative dehydratase | 5098–5526 | 39,256–39,684 | 429 | Forward | COL, CP000046 [39169:39597] and MW2, BA000033 [39128:39556] |
| <i>txbi_mecA</i> | Bidirectional rho-independent terminator of <i>mecA</i> | 5517–5581 | 39,675–39,739 | 65 | | |
| <i>mecA</i> | Penicillin binding protein 2a | 5572–7578 | 39,730–41,736 | 2007 | Reverse | MW2, BA000033.2 [39602:41608] |
| <i>mecR1</i> -trunc | Methicillin resistance operon repressor 1, truncated as in SCCmec I, IV, V | 7678–8652 | 41,836–42,810 | 975 | Forward (trunc.) | |
| <i>hsdR2</i> -IS1272 | Fragment of type I restriction-modification system endonuclease | 8653–8886 | 42,811–43,044 | 234 | Truncated | COL, CP000046 [42724:42957] and MW2, BA000033 [42683:42916] |
| tnpIS1272 | Transposase for IS1272 | 8887–10,410 | 43,045–44,568 | 1524 | Reverse | COL, CP000046 [42958:44481] and MW2, BA000033 [42917:44440] |
| Q9KX75 | Putative protein | 10,546–11,052 | 44,704–45,210 | 507 | Reverse | |
| Q7A207 | Putative protein | 11,068–11,379 | 45,226–45,537 | 312 | Reverse | 06BA18369, ARXY01000001 [134883:135194] |
| Q7A206 | Putative protein | 11,466–11,816 | 45,624–45,974 | 351 | Reverse | 06BA18369, ARXY01000001 [135281:135631] |
| <i>ccrB-1</i> | Cassette chromosome recombinase B, type 1 | 12,282–13,907 | 46,440–48,065 | 1626 | Reverse | |
| <i>ccrA-1</i> | Cassette chromosome recombinase A, type 1 | 13,929–15,278 | 48,087–49,436 | 1350 | Reverse | 06BA18369, ARXY01000001 [137744:139093] |
| <i>cch-1</i> | Cassette chromosome helicase | 15,466–17,235 | 49,624–51,393 | 1770 | Reverse | CMFT532, HF569114 [8453:10222] |
| DUF1413-06BA18369 | Putative protein associated with <i>cch</i> | 17,235–17,525 | 51,393–51,683 | 291 | Reverse | 06BA18369, ARXY01000001 [141050:141340] |
| Q83ZD5 | Putative protein | 17,696–18,769 | 51,854–52,927 | 1074 | Forward | 06BA18369, ARXY01000001 [141511:142584] |
| Helicase M06 | DEAD/DEAH box helicase domain protein | 18,863–20,803 | 53,021–54,961 | 1941 | Forward | 06BA18369, ARXY01000001 [142678:144618] |
| Q6GD51 | Putative protein | 21,060–21,368 | 55,218–55,526 | 309 | Forward | 06BA18369, ARXY01000001 [144875:145183] |
| D3QFP0-SCC | Putative lipase/protease | 21,415–21,653 | 55,573–55,811 | 239 | Reverse (trunc.) | 06BA18369, ARXY01000001 [145230:145468] and MSSA476, BX571857 [52281:52519] |
| Continued | | | | | | |

| Gene ID | Explanation | Position in SCC (nt) | Position in genome (nt) | Length (nt) | Direction | Sequence identical to |
|-----------------------|-----------------------------------|----------------------|-------------------------|-------------|------------------|---|
| D3JCW9 | Putative protein | 21,478–21,636 | 55,636–55,794 | 159 | Forward | 06BA18369, ARXY01000001 [145293:145451] and MSSA476, BX571857 [52344:52502] |
| <i>fusC</i> | Fusidic acid resistance protein C | 21,954–22,592 | 56,112–56,750 | 639 | Forward | 06BA18369, ARXY01000001 [145769:146407] and MSSA476, BX571857 [52820:53458] |
| tnpIS150 | Transposase of IS150 | 23,068–23,382 | 57,226–57,540 | 315 | Forward | CMFT463, HF569110 [16043:16357] |
| tnp_A8YYY6 | Transposase | 23,394–24,209 | 57,552–58,367 | 816 | Forward (trunc.) | |
| Q4LAG7-SCC <i>fus</i> | Putative protein | 24,356–24,784 | 58,514–58,942 | 429 | Reverse | 06BA18369, ARXY01000001 [148171:148599] |
| <i>yobV</i> | Transcriptional regulator | 24,864–25,793 | 59,022–59,951 | 930 | Forward | 06BA18369, ARXY01000001 [148679:149608] |
| DR_SCC | Direct repeat of SCC | 25,890–25,908 | 60,048–60,066 | 19 | | |

Table 1. The SCC*mec*/SCC*fus* composite element in M58.

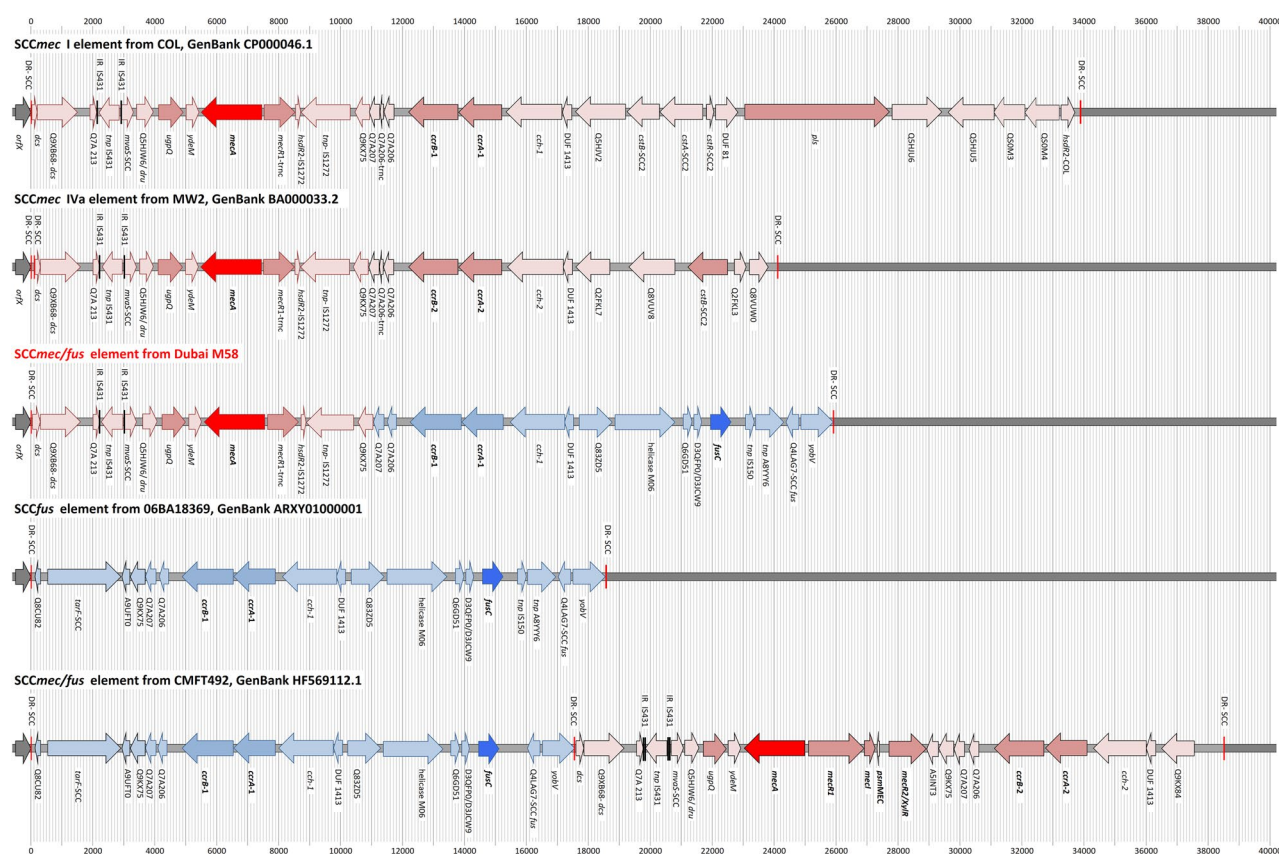


Figure 1. The SCC*mec*/SCC*fus* element in M58 in comparison to some reference sequences (COL, MW2, 06BA18369 and CMFT492).

CC1153-MRSA were mostly identified in Kuwait and the UAE. The clear majority, i.e. all isolates except the oldest one³³, harboured SCC*mec*/SCC*fus* chimeric elements and the most common variant that could be described either as SCC*mec* I + *fusC* element or as a pseudoSCC*mec* class B + *fusC* + *ccrA*/B-1 element was sequenced. Sequence analysis also revealed (see above and Table 1) that *ccrB*-1 and accompanying genes are more related to alleles from other SCC*fus* elements rather than to the ones from SCC*mec*. Thus, a description as a pseudoSCC*mec* class B + *ccrA*/B-1 + *fusC* element should be regarded as the correct one. The *mec* complex B could have been derived from either a SCC*mec* I or SCC*mec* IV element. However, the latter one was more probable based on of the MW2-like allele of *mecA*.

The entire region associated with *fusC* (encompassing Q7A207, Q7A206, *ccrB*-1, *ccrA*-1, *cch*-1, DUF1413, Q83ZD5, helicase M06, Q6GD51, D3QFP0-SCC, D3JCW9, *fusC*, tnpIS150, tnp_A8YYY6, Q4LAG7-SCC*fus* and *yobV*) could be seen as one mobile genetic element that got introduced into a CC1153-MRSA replacing Q7A207, Q7A206 and the *ccr* recombinase genes that previously belonged to its SCC*mec* element. This set of genes was also

found in a *Staphylococcus hominis* subsp. *hominis* strain and a CC5-MSSA from Canada (06BA18369 GenBank ARXY00000000.1) as described above. Furthermore, MRSA strains from Saudi Arabia²⁵ (as represented by isolates CMFT492, HF569112.1 and CMFT532, GenBank HF569114.1; see Tables 2, 3, 4 and 5) also carried the same region associated with *fusC* (differing from the one in M58 only in minor random deletions) as part of complex chimeric SCCmec II elements. In CMFT492, this cluster was inserted between *orfX* and a truncated SCCmec II element that lacked the *kdp* locus, *cstA/B/R* and the transposons introducing *ble/aadD* and *erm(A)/ant9*. In CMFT532 and other strains (see Tables 2, 3, 4 and 5), the region associated with *fusC* was inserted between *orfX* and a normal SCCmec II element. In these strains, additional markers (sccterm13, Q8CU82, *tarF*-SCC, A9UFT0, Q9KX75) were also associated with the *fusC* element that were absent in M58. Furthermore, there were also strains such as FORC 090, GenBank CP029198.1 or AR466, CP029080.1 in which *fusC* and its immediate neighbours (Q83ZD5, helicase M06, Q6GD51, D3QFP0-SCC, D3JCW9, tnpIS150, tnp A8YYY6, Q4LAG7-SCC*fus* and *yobV*) were accompanied by other *ccr* recombinase genes and other genes upstream, towards *orfX*. This prompted us to review published sequences and to compare them with the CC1153 strain described herein to sort and to classify the different gene clusters accompanying *fusC*.

Review of *fusC* elements. When comparing the region around *fusC* from M58 to published sequences, it became obvious that 64 published *S. aureus* sequences (plus three *S. hominis* sequences and nine un-assembled *S. aureus* sequences from the Short Read Archive) cluster into 31 different SCC*fus* or SCCmec/*fusC* chimeric or composite elements (Tables 2, 3, 4, 5/Supplemental File 5). None of these fully matched the M58 sequence. When considering only the immediate region around *fusC*, strains were identified in which the same gene cluster as M58 was present and it was observed that there are only four, possibly five, different sets of genes directly accompanying this resistance gene (Tables 2, 3, 4, 5/Supplemental File 5 and Fig. 2).

These sets could be regarded as fixed gene complexes in analogy to the *mec* complexes A (in SCCmec II and III), B (in SCCmec I and IV) and C (in SCCmec V). Likewise, they are also associated with different sets of SCC-recombinase genes including alleles of *ccrA*-1/*ccrB*-1, *ccrA*-1/*ccrB*-3, *ccrA*-3/*ccrB*-3, *ccrA*-4/*ccrB*-4, *ccrA*/*ccrB*1 (FORC_90) and *ccrAA/ccrC*. Resulting “SCC*fus*” elements can, besides *fusC*-complexes and recombinase genes, also carry additional payload including *tarF* (teichoic acid biosynthesis protein F), *speG* (spermidine N-acetyltransferase), various variants of type I restriction-modification systems or *mec* complexes. They also can be linked to entire SCCmec elements resulting in complex genomic islands sometimes even including multiple sets of recombinase genes. These additional components can be localized upstream (towards *orfX*) or downstream (see Tables 2, 3, 4, 5/Supplemental File 5).

The longest known of the *fusC*-complexes (“A”, Table 2, see also Supplemental File 5 and Fig. 2), as in MSSA476, BX571857.1, comprises Q6GD54 (putative protein), Q6GD53 (putative protein), *tirS* (staphylococcal Toll/interleukin-1 receptor domain mimic), Q6GD51, D3QFP0-SCC, D3JCW9, *fusC*, sccterm03, Q6GD49 (putative protein), Q8CU43 (putative protein), Q4LAG7-SCC*fus* and *yobV* (for explanations and GenBank entries of the genes discussed, see Supplemental File 4). It can be found in MSSA, such as the prototypical CC1-MSSA sequence Sanger MSSA476, as well as in MRSA. It appears in MRSA strains (CC1 and CC5) with SCCmec IV elements, mainly from the Middle East^{25, 28, 30, 31}, Australia and New Zealand³⁸ as well as in SCCmec V strains from the Middle East.

A second *fusC*-complex (“B”, Table 3, see also Supplemental File 5 and Fig. 2) comprises Q6GD54, Q6GD53, *tirS*, Q6GD51, D3QFP0-SCC, D3JCW9 and *fusC*. Besides gene content, it also differs from all others in five characteristic single nucleotide polymorphisms (SNP) within the *fusC* gene (14A > C; 150T > G; 290G > C; 537A > T; 632T > C). This complex has apparently not yet been observed in MSSA but there are several MRSA strains harbouring it connected to various SCCmec elements. One is HDE288, as prototypical sequence the “New Paediatric” CC5-MRSA strain from Portugal⁴⁷. Here, the *fusC*-complex is accompanied by *ccrA/B*-4 genes and a *mec* complex B, a combination also referred to as SCCmec VI. Another CC5 (ST149) strain, known from Malta⁴⁸, the Middle East^{14, 25} and UK²⁶ harbours the same *fusC*-complex, together with *ccrA/B*-3 alleles and a SCCmec IV element. It also appears, although Q6GD54 is absent, in a SCCmec I MRSA strain from France (CC5, “Geraldine Clone”⁴⁹).

A third *fusC*-complex (“C”, Table 4, see also Supplemental File 5 and Fig. 2) consisting of Q83ZD5, helicase M06, Q6GD51, D3QFP0-SCC, D3JCW9, *fusC*, tnpIS150, tnp A8YYY6, Q4LAG7-SCC*fus* and *yobV*. The *fusC* sequence is identical to the one in MSSA476, BX571857.1. This is the variant found in M58 and the other strains discussed above. In these strains, it is accompanied by a largely identical set of recombinase-associated genes. The carriage of sccterm13, Q8CU82, *tarF*-SCC, A9UFT0, Q9KX75 as additional payload is variable; in M58 these genes are replaced by the *mec* complex, while in the CMFT492, CMFT535 etc., the SCCmec element is located downstream away from *orfX*²⁵. Strains FORC090 (CP029198.1), AR466 (CP029080.1), MRSA18 (SAMEA1317993)²⁶ and 20121643 (ERR1595888/SAMEA3924203) harbour the same *fusC*-complex but in these sequences, it is accompanied by other recombinase alleles.

A fourth *fusC*-complex (“D1”, Table 5, see also Supplemental File 5 and Fig. 2) consists of Helicase M06, Q6GD51, D3QFP0-SCC, D3JCW9, *fusC*, sccterm03, Q6GD49, Q8CU43, Q4LAG7S-SCC*fus* and *yobV*. Its *fusC* gene has one characteristic SNP (309G > A). It was not yet found in MSSA, but in MRSA belonging to CC8, CC30 and ST834³⁰. In these strains, it is accompanied by a *mec* complex B and a set of *ccrA/B*-4 genes. It has been found neither in any other context, nor in MSSA strains.

A CC15 SCCmec V MRSA strain harbours a *fusC*-complex consisting of helicase M06, Q6GD51, D3QFP0-SCC, D3JCW9, *fusC*, sccterm03, Q6GD49 and Q8CU43. The *fusC* sequences contain a specific SNP (486T > C), but otherwise, gene content (apart from the lack of Q4LAG7-SCC*fus* and *yobV*) and the order of genes are the same as in the fourth complex (hence, “D2”, Table 5, see also Supplemental File 5 and Fig. 2). The *fusC*-complex itself is in all these sequences localised on one contig, but other associated markers such as SCCmec, *orfX* etc. are split across several contigs. One ST72 sequence (HST-084, AZTF00000000.1) has a SCCmecV/SCC*fus* chimeric

| Reference Sequence: Strain (Clonal Complex), GenBank accession | Other SCC markers upstream/towards from <i>orfX</i> | <i>fusC</i> -associated recombinases | <i>fusC</i> -associated complex | Other SCC markers downstream/away from <i>orfX</i> | Other genome sequences harbouring the same SCC/ <i>fus</i> element | Other references or descriptions and geographic background |
|--|--|--------------------------------------|---------------------------------|--|--|---|
| MSSA476 (CC1) , BX571857.1 | sccterm06 (terminus of SCC towards <i>orfX</i>), <i>hsdR</i> (type I restriction-modification system, TIRMS, endonuclease), <i>hsdS</i> (TIRMS site-specificity determinant), <i>hsdM</i> (TIRMS DNA methylase) | <i>ccrB-1</i> , <i>ccrA-1</i> | A | N/A | FORC_026 (CC1), CP013132.1; FORC_045 (CC1), CP017115.1; FDAARGOS_2 (CC1), CP026964.1; PMB_81-4 (CC1), CP034441.1; KT314250 (CC1), AOCIP; MUF256, AZSE; Strain 21304, JHPW; ST20110167 (CC1), FSSD; 3 (B) GCID_STAPE_0004, SUKH; 23 GCID_STAPE_0022, SUKK; 43 GCID_STAPE_0029, SUKO; 3 (A) GCID_STAPE_0003, SUKX; 6 (A) GCID_STAPE_0004, SUKY | CC1-MSSA from France ³⁵ , India ⁴⁰ , Malaysia ⁴¹ , South Korea, UK ⁴² , and the USA (?) Bovine CC1-MSSA from South Korea <i>Note</i> : this corresponds to "SCC476" ²⁶ . |
| WMCS6087 , JBHR | sccterm06, <i>hsdR3</i> , <i>hsdS3</i> , <i>hsdM3</i> | <i>ccrB-1</i> , <i>ccrA-1</i> | A** | SCCmec II | N/A | Isolate from the USA |
| Strain 515798 (CC1) , CP045474.1 | SCCmec IV a, sccterm06, <i>hsdR3</i> , <i>hsdS3</i> , <i>hsdM3</i> | <i>ccrB-1</i> , <i>ccrA-1</i> | A | N/A | N/A | MRSA 2.3, 6, 14, 22 from UK ²⁶ ; CC1-MRSA from UAE ^{39,43,44} <i>Note</i> : this corresponds to "SCC476 and SCCmec IVa" and "SCCmec-fus I" ²⁶ . |
| NZAK3 (CC5) , LT009690.1 | SCCmec IV a | N/A | A | N/A | N/A | CC5-MRSA from New Zealand ³⁸ ; MRSA 10 (CC5) from UK ²⁶ <i>Note</i> : this corresponds to "SCCmec-fus III" ²⁶ . |
| MRSA3 ST20121850 (CC1) , FSRZ | SCCmec V | <i>ccrB-1</i> , <i>ccrA-1</i> | A | N/A | N/A | CC1-MRSA from France ³⁵ |
| MUM475 (CC1) , AZSG** | SCCmec VT, sccterm06*, <i>hsdR3</i> , <i>hsdS3</i> , <i>hsdM3</i> | <i>ccrB-1</i> , <i>ccrA-1</i> | A | N/A | MRSA1_ST20130096 (CC1), FSRY | CC1-MRSA from India ⁴⁰ ; CC1-MRSA from France ³⁵ |
| TFGsh1 (<i>S. hominis</i>) , AB930126.1 | sccterm07, <i>hsdR</i> , <i>hsdS</i> , <i>speG</i> | <i>ccrB-1</i> , <i>ccrA-1</i> | A | N/A | N/A | <i>S. hominis</i> subsp. <i>hominis</i> from Taiwan ⁴⁵ |
| NTUH-4729 (CC239) , KF527883.1 | sccterm07, <i>hsdR</i> , <i>hsdS</i> , <i>speG</i> | <i>ccrB-1</i> , <i>ccrA-1</i> | A | SCCmec III | N/A | CC239-MRSA from Taiwan ⁴⁶ |
| 45394F , GU122149.1 | sccterm07, <i>hsdR</i> , <i>hsdS</i> , <i>speG</i> | <i>ccrB-1</i> , <i>ccrA-1</i> | A | SCCmec IV | N/A | MRSA from the Netherlands (?) |
| MSSA 199 (CC8) , CP031667.1 | <i>des-trnc</i> , sccterm07, <i>hsdR-trnc</i> , <i>hsdS</i> | <i>ccrB-3</i> , <i>ccrA-1</i> | A | N/A | MSSA 64 (CC8), CP031670.1; UP_1591 (CC8), CP047809.1; ST20121341 (CC8), FSSE | CC8-MSSA from Germany; CC8-MSSA from France ³⁵ |
| CMFT535 (CC22) , HF569115.1 | sccterm07, <i>hsdR-trnc</i> , <i>hsdS</i> | <i>ccrB-3</i> , <i>ccrA-1</i> | A | SCCmec IV | CMFT201 (CC22), HF569098.1; CMFT303 (CC22), HF569103.1; CMFT306 (CC22), HF569104.1 | CC22-MRSA from Saudi Arabia ⁴³ |

*variably present in different sequences of the respective type, **split across two or more contigs so that the correct order of SCC elements and/or individual genes cannot be determined

Table 2. Staphylococcal Cassette Chromosomes with a *fusC*-associated complex **A** consisting of Q6GD54, Q6GD5, *tirS*, Q6GD51, D3QFP0-SCC, D3JCW9, *fusC*, sccterm03, Q6GD49, Q8CU43, Q4LAG7-SCC*fus* and *yobV*. Please note that this is an abridged version (with genes encoding "putative proteins" omitted); for a more detailed version, see Supplement 5. References^{25, 26, 35, 38–46}. *Variably present in different sequences of the respective type. **Split across two or more contigs so that the correct order of SCC elements and/or individual genes cannot be determined.

| Reference Sequence: Strain (Clonal Complex), GenBank accession | Other SCC markers upstream/towards from <i>orfX</i> | <i>fusC</i> -associated recombinases | <i>fusC</i> -associated complex | Other SCC markers downstream/away from <i>orfX</i> | Other genome sequences harbouring the same SCC/ <i>fus</i> element | Other references or descriptions and geographic background |
|--|---|--------------------------------------|---------------------------------|--|--|--|
| CMFT3002 (CC5) , HF569102.1 | sccterm14, <i>yeeA</i> (putative DNA methyltransferase) | <i>ccrB-3</i> , <i>ccrA-3</i> | B | SCCmec IV | CMFT106 (CC5), HF569093.1; CMFT181 (CC5), HF569097.1; SAMEA2042721 | Maltese CC5 isolates ⁴⁸ ; CC5-MRSA from Saudi Arabia ^{14,25} ; MRSA 1, 4, 5, 13, 15, 17 from UK ²⁶ . <i>Note</i> : this corresponds to "SCCmec IVa and SCC148" ²⁶ . |
| ERS1461791/DSM 28763 (CC8) , LT671859.1 | SCCmec IV | N/A | B | N/A (in CC45 sequences, a CC45 specific genomic island is directly adjacent: CP006044.1 CA347_83 to 88) | MRSA2_ST20120331 (CC5), FSRW SAMEA1317980 (CC45) SAMEA2664472 (CC45) SAMEA2664988 (CC45) | CC8-MRSA from Germany (LT671859.1), CC5-MRSA from France ³⁵ MRSA8, 9, 11, 12, 16, 19, 20, 21, 23 (CC45) from UK ²⁶ Probably (based on array profile) an epidemic CC22-MRSA from Germany ⁵⁰ <i>Note</i> : this corresponds to "SCCmec-fus IV" ²⁶ . |
| Lib-655 (CC45) , SAMEA104188236 | SCCmec IV | N/A | B | <i>hsdM/hsdS/hsdR</i> and the CC45- specific genomic island | N/A | CC45-MRSA from Germany |
| HDE288 (CC5) , AF411935.3 | [<i>mec</i> complex B] | <i>ccrB-4</i> , <i>ccrA-4</i> | B | N/A | FDAARGOS_48 (CC5), CP026953.1; FDAARGOS_1 (CC5), CP026968.1; 091751_57, GenBank AHZH/ SAMN02470727 | CC5-MRSA from Portugal ^{30,55} ; MRSA from Switzerland; UK-EMRSA-12 and -13 (CC8) from UK |
| NCTC13140 (CC8) , LS483319.1 | [<i>mec</i> complex B] | <i>ccrB-4</i> , <i>ccrA-4</i> | B | N/A | N/A | CC8-MRSA from UK |
| HT20030749 (CC5) , FSRX | SCCmec I with an integrated plasmid harbouring <i>aadD</i> and <i>ble</i> | N/A | B | <i>hsdR</i> , <i>hsdM</i> | SAMEA2664142 (CC5) | Geraldine Clone from France ^{35,49} ; MRSA 7 from UK ²⁶ <i>Note</i> : this corresponds to "SCCmec-fus II" ²⁶ . |

Table 3. Staphylococcal Cassette Chromosomes with a *fusC*-associated complex **B** consisting of Q6GD54, Q6GD53, *tirS*, Q6GD51, D3QFP0-SCC, D3JCW9 and *fusC* (14A > C; 150 T > G; 290G > C; 537A > T; 632 T > C). Please note that this is an abridged version; for a more detailed version, see Supplement 5. References^{14, 25, 26, 35, 47–51}.

| Reference Sequence: Strain (Clonal Complex), GenBank accession | Other SCC markers upstream/towards from <i>orfX</i> | <i>fusC</i> -associated recombinases | <i>fusC</i> -associated complex | Other SCC markers downstream/away from <i>orfX</i> | Other genome sequences harbouring the same SCC/ <i>fus</i> element | Other references or descriptions and geographic background |
|--|--|--------------------------------------|---------------------------------|---|--|--|
| 06BA18369 (CC5), ARXY | sccterm13, <i>tarF</i> -SCC | <i>ccrB-1, ccrA-1</i> | C | N/A | <i>Staphylococcus hominis</i> subsp. <i>hominis</i> NTUH-339, GenBank KY643657.1 | CC5-MSSA from northern Saskatchewan, Canada ⁵³ <i>S. hominis</i> subsp. <i>hominis</i> from Taiwan |
| CMFT532 (CC30), HF569114.1 | sccterm13, <i>tarF</i> -SCC | <i>ccrB-1, ccrA-1</i> | C | SCCmec II | CMFT2 (CC30), HF569101.1; CMFT120 (CC30), HF569094.1; CMFT151 (CC30), HF569095.1; CMFT283 (CC30), HF569100.1; CMFT352 (CC30), HF569107.1; CMFT463 (CC30), HF569110.1; CMFT489 (CC30), HF569111.1 | CC30-MRSA from Saudi Arabia ²⁵ |
| CMFT492 (CC30), HF569112 | sccterm13, <i>tarF</i> -SCC | <i>ccrB-1, ccrA-1</i> | C | Truncated SCCmec II | N/A | CC30-MRSA from Saudi Arabia ²⁵ |
| M58 | [<i>mec</i> complex B] | <i>ccrB-1, ccrA-1</i> | C | N/A | N/A | This paper |
| FORC 090 (CC5), CP029198.1 | <i>yeeA</i> , transposase for IS256, nuclease, <i>YeeC</i> -like putative protein | <i>ccrB, ccrA</i> | C | SCCmec II | N/A | South Korea |
| MRSA18 (CC8), SAMEA1317993 | sccterm07, <i>hsdR, hsdS, ccrB-4, ccrA-4, mec</i> complex B | <i>ccrB-4, ccrA-4</i> | C | N/A | N/A | MRSA 18 from UK ²⁶ |
| St960 (CC227), FGH8**/ SAMEA1317550 | sccterm07, <i>hsdR</i> | <i>ccrB-4, ccrA-4</i> | C | SCCmec IV | N/A | CC22 (?), described as UK-EMRSA-15) from UK |
| AR466 (CC45), CP029080.1 | sccterm07, <i>hsdR, hsdS, ccrB-4, ccrA-4, thyA-pla, dfrA, IS431, mec</i> complex B | <i>ccrB-4, ccrA-4</i> | C | N/A | N/A | CC45-MRSA from the USA |
| Strain 20121643 (CC8), ERR1595888/SAMEA3924203 | sccterm07, <i>hsdR, hsdS, ccrB-4, ccrA-4, thyA-pla, dfrA, IS431, mec</i> complex B | <i>ccrB-4, ccrA-4</i> | C | <i>mupC2-SCC, psuG-SCC, ccrA-4</i> | N/A | CC8-MRSA, no details specified, from "European Region". |
| ER03364.3 (CC72), CP030550.1 | SCCmec VT with integrated <i>tetK</i> | N/A | (Truncated C) | N/A | N/A | CC72 MRSA from the USA |
| M06/0171 (CC779), HE980450.1 | IS431, <i>mec</i> complex C, IS431 | N/A | (Truncated C) | Copper resistance operon, <i>speG, ccrB-4, ccrA-4, ccrAA, ccrC, hsdR, cas</i> | N/A | CC779-MRSA from Ireland ⁵² |
| TFGsh5-1 (<i>S. hominis</i>), AB930128.1 | sccterm02, IS431 | N/A | (Truncated C) | <i>speG</i> , arsenic resistance operon | N/A | <i>S. hominis</i> subsp. <i>hominis</i> from Taiwan ⁴⁵ |

**split across two or more contigs so that the correct order of SCC elements and/or individual genes cannot be determined

Table 4. Staphylococcal Cassette Chromosomes with a *fusC*-associated complex C consisting of Q83ZD5, helicase M06, Q6GD51, D3QFP0-SCC, D3JCW9, *fusC*, tnpIS150, tnp A8YYY6, Q4LAG7-SCC*fus* and *yobV*. Please note that this is an abridged version; for a more detailed version, see Supplement 5. References^{25, 26, 45, 52, 53}. **Split across two or more contigs so that the correct order of SCC elements and/or individual genes cannot be determined.

| Reference Sequence: Strain (Clonal Complex), GenBank accession | Other SCC markers upstream/towards from <i>orfX</i> | <i>fusC</i> -associated recombinases | <i>fusC</i> -associated complex | Other SCC markers downstream/away from <i>orfX</i> | Other genome sequences harbouring the same SCC/ <i>fus</i> element | Other references or descriptions and geographic background |
|--|---|--------------------------------------|---------------------------------|---|--|--|
| RUH-32 (CC30), MK991791.1 | <i>mec</i> complex B | <i>ccrB-4, ccrB-4</i> | D1 | N/A | SAMEA2385458 (CC8), SAMEA2385540 (CC8), SAMEA2664046 (CC8), SAMEA2664096 (CC8) | CC8-MRSA from Germany/Middle East ³⁰ ; CC30-MRSA from Kuwait, Saudi Arabia and UAE ³⁰ ; ST834-MRSA from Saudi Arabia ³⁰ |
| RUH-2 (CC15), MF185204.1 to -09.1** | sccterm10, F8WKf9, F8WKG0, ALS84360, Q7A213, IS431 ** | N/A | D2 | SCCmec V with integrated plasmid harbouring <i>aacA-aphD</i> ** | RUH-71 (CC15), NHZW RUH-98 (CC15), NHZW RUH-99 (CC15), NHZX | Human and livestock CC15 MRSA from Saudi Arabia ^{28,34} ; CC15-MRSA from Kuwait ³² Livestock CC15 MRSA from Egypt (author's unpublished observation) |
| HST-084 (CC72), AZTF | (SCCmec V)** | ** | (Truncated D2) | <i>ccrAA</i> | N/A | CC72 MRSA from Lebanon |

**split across two or more contigs so that the correct order of SCC elements and/or individual genes cannot be determined

Table 5. Staphylococcal Cassette Chromosomes with a *fusC*-associated complex D consisting of Helicase M06, Q6GD51, D3QFP0-SCC, D3JCW9, *fusC* (309G > A), sccterm03, Q6GD49, Q8CU43, Q4LAG7-SCC*fus* and *yobV* (**D1**) or Helicase M06, Q6GD51, D3QFP0-SCC, D3JCW9, *fusC* (486 T > C), sccterm03, Q6GD49, and Q8CU43 (**D2**). Please note that this is an abridged version; for a more detailed version, see Supplement 5. References^{28, 30, 32, 34}. **Split across two or more contigs so that the correct order of SCC elements and/or individual genes cannot be determined.

element harbouring Helicase M06, Q6GD51, D3QFP0-SCC, D3JCW9 and a *fusC* gene with the same (486T > C) SNP but unfortunately, it is fragmented across several contigs. Another strain (ER03364.3, CP030550.1) with a SCCmecVT/SCC*fus* chimeric element also harbours helicase M06, Q6GD51, D3QFP0-SCC, D3JCW9 and *fusC*. However, the identity of *fusC* with the one in MSSA476, BX571857.1 (i.e., the absence of the 309G > A SNP) suggests it to be derived from the third *fusC*-complex (Table 4, see also Supplemental File 5).

Further investigations on *fusC*-complexes associated with SCCmec V/VT are warranted as such isolates from diverse clonal complexes including CC5, CC97, CC121 and CC1153 (see above) have been observed, especially in the Arabian Gulf region.

Finally, truncated *fusC*-complexes were also observed as part of a very complex composite SCCmec element in a CC779 isolate M06/0171, HE980450.1⁵² and of a composite "pseudo-SCC" element (i.e., without *ccr* genes) in *Staphylococcus hominis* subsp. *hominis* TFGsh5-1, AB930128.1. As the third and the fourth complex, it encompasses genes for helicase M06, Q6GD51, D3QFP0-SCC, D3JCW9 and *fusC*; but its *fusC* allele indicates relation to the third one (Table 4, see also Supplemental File 5).

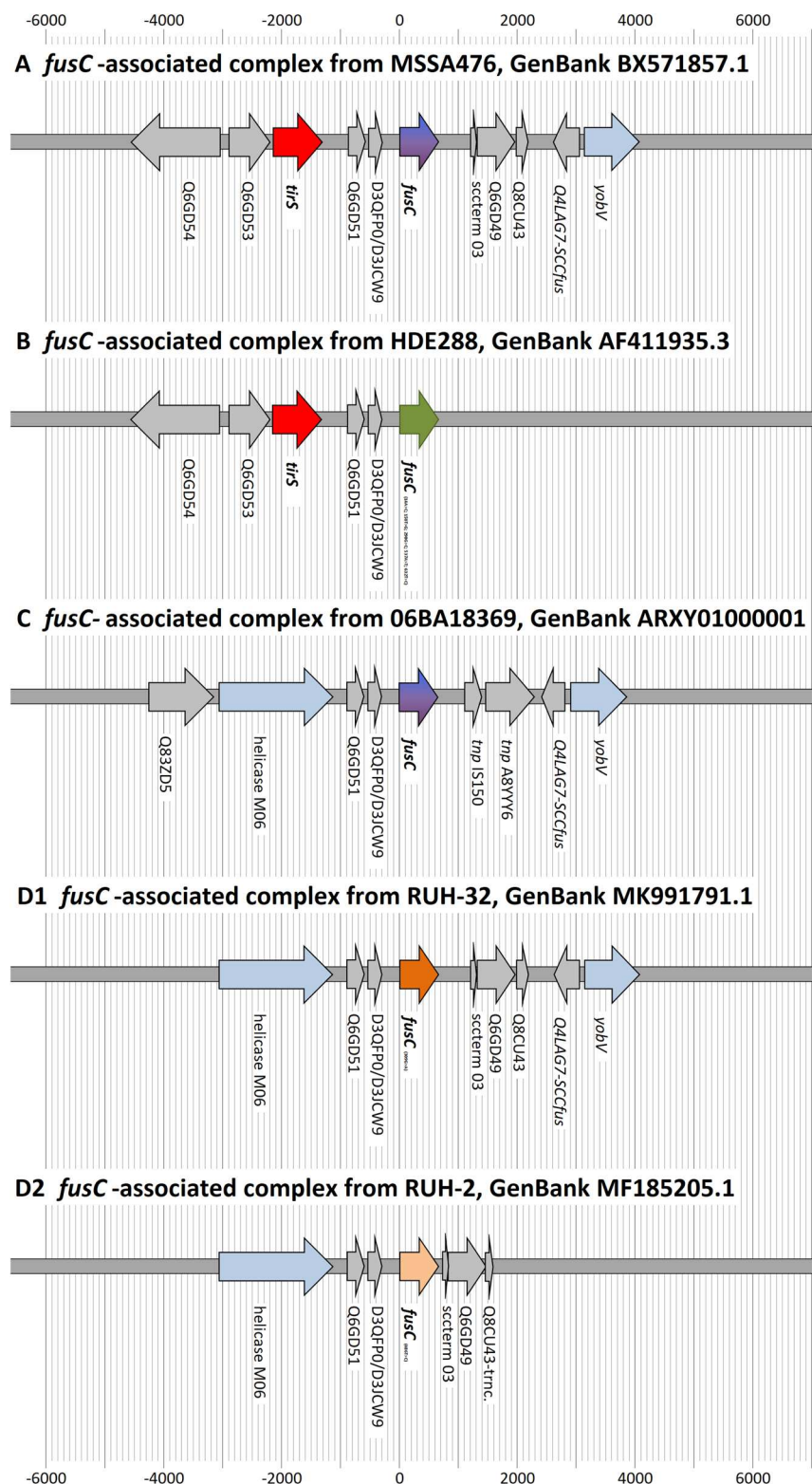


Figure 2. The different sets of genes directly accompanying *fusC* in M58 and in previously published SCC*fus* or SCC*mec/fus* elements.

There was no obvious phenotypical correlation of *fusC*-complexes to fusidic acid MICs, all tested strains (Supplemental File 6) were highly resistant regardless of their actual type of *fusC*-complex.

Further open questions are the timeframe of the evolution of SCC*fus* elements as well as their geographical origins. A wide variety of *fusC*-positive strains has not yet been sequenced. It would be interesting to know whether additional elements exist, and whether there are MSSA strains harbouring those *fusC*-complexes yet observed in MRSA only. The origin and evolutionary history of *tirS* is another open question. This virulence factor³⁵ is present in two out of five *fusC*-complexes but to the best of our knowledge, it has never been observed in another context.

As previously discussed³⁰, *fusC* was detected in as much as twenty-two different clonal complexes of *S. aureus*, CC1, CC5, CC6, CC7, CC8, CC15, CC22, CC30, CC45 [*agr* I], CC45 [*agr* IV], CC50, CC59, ST72, CC88, CC97, CC121, CC152, CC361, CC779, ST834, CC913 and CC1153 from essentially all parts of the world. This and the emergence of *fusC*-MRSA especially in the Middle East indicate a selective advantage associated with its presence. Fusidic acid can be administered intravenously, but this is not commonly done, and the intravenous formulation is not available everywhere. It is also used topically, as ointment for presumably staphylococcal skin conditions. Observations from New Zealand suggest a quick emergence of *fusC*-positive *S. aureus* in parallel to an increasing use of this compound^{16,38}. A co-evolution of SCC*mec* and SCC*fus* elements might result in a public health hazard, as strains with composite or chimeric elements are selected for both, in the hospital by beta-lactam administration as well as in the community by topical use of fusidic acid. Thus, antibiotic stewardship and infection control measures targeting MRSA in the hospital must be accompanied by restrictions to an uncontrolled over-the-counter sale of fusidic acid in outpatient settings as well as by a prudent use in outpatient settings.

Materials and methods

Isolates. A list of the isolates studied is provided in Supplement 1. Isolates were selected out of various typing and epidemiological projects based on array hybridisation profiles indicative for an affiliation to CC1153.

The CC1153-MRSA from the UAE were isolated from skin and wound infections. The CC1153-MRSA from Saudi Arabia also originated from a wound infection. One CC1153-MSSA from Saudi Arabia was a nasal colonizer from a healthcare worker, the others originated from skin and soft tissue infections (with three of them being identified during an earlier study⁵⁴). The first CC1153-MRSA reported in Kuwait was cultured from a wound inflicted by a dog bite in 2009³³. The other isolates were obtained between 2017 and 2020 mostly from wound infections of patients located in six hospitals. One isolate was isolated from a gynaecological swab, one from a nasal swab and one was obtained from blood culture. Egyptian isolates were identified from rural small-holder dairy cattle that showed sub-clinical mastitis, i.e., somatic cell counts > 200,000 cell/mL and positive results of California Mastitis Test. The isolates were collected from six different cows in a herd, consisting of 25 crossbred dairy cows, located at Dakahlia Governorate in the northeast of Cairo, Egypt. The milking procedure was performed manually in the examined cows, while the medical records of the farm revealed the usage of a wide spectrum of antibacterial agents³⁷. French isolates (11 MSSA, 1 MRSA) had been isolated during infections (cutaneous (n = 8), respiratory (n = 3), blood culture (n = 1)) in ten different hospitals between 2010 and 2017. One German MRSA isolate was cultured from an abscess of an approximately half year-old Egyptian child whose family lives in Germany. One German MSSA isolate originated from an abscess.

PVL detection was performed on six isolates by an experimental lateral flow test⁵⁴. Fusidic acid MICs were determined by agar gradient dilution tests with commercially available strips (01B10122 Fusidinsäure MIC Test Strip 0.016–256 µg/mL, Bestbion dx GmbH, Cologne, Germany).

Microarray-based molecular characterization. Genotyping of all strains was performed using the *S. aureus* Genotyping Kit 2.0 system (Abbott [Alere Technologies GmbH, Jena, Germany]) microarray-based assay. The array covers 333 different targets related to approximately 170 different genes and their allelic variants. The list of target genes as well as sequences of probes and primers have previously been published along all relevant protocols^{9,39,55}.

Staphylococcus aureus was cultivated on Columbia blood agar. The DNA extraction was performed using lytic enzymes (lysostaphin, lysozyme, RNase) and buffer from the *S. aureus* Genotyping Kit 2.0 kit and Qiagen DNA extraction columns (Qiagen, Hilden, Germany) according to manufacturers' instructions. Then, a linear amplification was performed using one primer for each target sequence. During the linear multiplex-amplification, biotin-16-dUTP was incorporated into the amplicons, which were then stringently hybridised to the specific probes on the microarray. After washing steps, hybridisation was detected using streptavidin horseradish peroxidase that triggered local precipitation at those spots where amplicon was bound. Microarrays were photographed and analysed with a designated reader and software (IconoClust, Abbott [Alere Technologies]). Analysis allowed detecting presence or absence of certain genes or alleles, as well as assignment to the clonal complex, strains, and SCC*mec* types.

Whole-genome sequencing. Genomic DNA was isolated from an overnight culture grown at 37 °C on Columbia blood agar using a Macherey and Nagel NucleoSpin Microbial DNA kit (MACHEREY-NAGEL GmbH & Co. KG, Dueren, Germany).

The Nanopore Oxford MinION platform was used for sequencing the whole genome of the CC1153 isolate M58 from the UAE. Briefly, size selection was performed using AMPure beads in a ratio 1:1 (v/v) with the DNA sample. The DNA library was generated using the nanopore sequencing kit SQK-LSK109 and the native barcoding expansion kit EXP-NBD103 (Oxford Nanopore Technologies, Oxford, UK) according to manufacturer's instructions. The used flowcell FLO-MIN106 (R9-Version) was primed by the flow cell priming kit EXP-FLP001 (Oxford Nanopore, Oxford, UK). The protocol named "1D Native barcoding genomic DNA" was used in version

NBE_9065_v109_revB_23May2018 (Last update: 03/09/2018). The guppy basecaller (v4.4.2., Oxford Nanopore Technologies, Oxford, UK) translated and trimmed the MinION raw data (fast5) into quality tagged sequence reads (4000 reads per fastq-file). Flye (v2.8.1) was used to assemble all reads to one large contig. Then, a racon-medaka (racon v1.4.3; medaka v1.2.0) pipeline was applied for polishing (with settings and descriptions being provided as Supplement 7).

The genome sequence is provided under GenBank accession number CP065857.1.

Phylogenetic analysis. A panel of 154 non-motile, core genomic markers was selected. Inclusion criteria were presence in all *S. aureus* clonal complexes analysed as well as uniform length in all genomes. The used genes as well as the genome sequences considered are listed in Supplemental File 3. Sequences were concatenated and analysed using SplitsTree 4.0⁵⁶ using default settings (Supplemental File 3).

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Author contributions

S.M., E.M., S.D.B. and R.E. wrote the manuscript drafts, M.A.P., M.B., S.B., M.E.A., M.G., R.N., H.H., A.S., A.M.S. and E.U. provided isolates and their initial identifications as well as background information. E.M., M.B., S.B., D.G., A.R., R.N. and A.R.L. did array experiments, S.D.B. and I.E. performed sequencing and initial analysis. S.M. analysed sequences and reviewed published sequences. All authors read, revised and approved the manuscript.

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Competing interests

IE is employee of BLINK AG, Jena, Germany. She provided crucial advice and supervision regarding Nanopore sequencing, as an experienced user of that technology but without having commercial affiliations or connections to Oxford Nanopore. DG is employee of PTC—Phage Technology Center GmbH, Hönen, Germany. He performed experiments for this study before being employed by this company. Thus, both commercial entities had no influence on the decision to publish and on the content of the study. All other authors declare no competing interests.

Additional information

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